

Genotyping protocol

General information:

Strain name	APC
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Primers:

Name	Sequence	Primer type
Lox13 LacZ Fw	CGTCGTTTTACAACGTCGTGAC	Selection cassette
Lox13 LacZ Rev	TAACAACCCGTCGGATTCTC	Selection cassette
Lox13 wt F2	CAGAAAAACGCCACAGGGGAGACG	Gene-specific
Lox13 wt R1	GCCTCACTCGGCCTTGAGGCTAATC	Gene-specific

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
WT Lox13	Lox13 wt F2	Lox13 wt R1
MUT (KO) Lox13 ^{LacZ}	Lox13 LacZ Fw	Lox13 LacZ Rev

Reaction mix:

Genomic dna (tail)	2	μl
Oligo Fw 10 pmol/μl	1	μl
Oligo Rev 10 pmol/μl	1	μl
REExtract-N-Amp™ PCR ReadyMix (SIGMA)	10	μl
H2O	6	μl
Final volume	20	μl

PCR program:

94 °C	1 min	X25
94 °C	30 sec	
63 °C	30 sec	
72 °C	30 sec	
72 °C	10 min	

Expected fragment size:

wt	400	bp
mutant	345	bp

Comments/Additonal information:

The PCRs are done independently (for Loxl3 wt and for LacZ alleles).